Supplement Material to „Improved PM_{10} Exposure Attenuates Age-Related Lung Function Decline: Genetic Variants in p53, p21 and CCND1 Modify this Effect"

Improved PM_{10} Exposure Attenuates Age-Related Lung Function Decline: Genetic Variants in *p53*, *p21* and *CCND1* Modify this Effect

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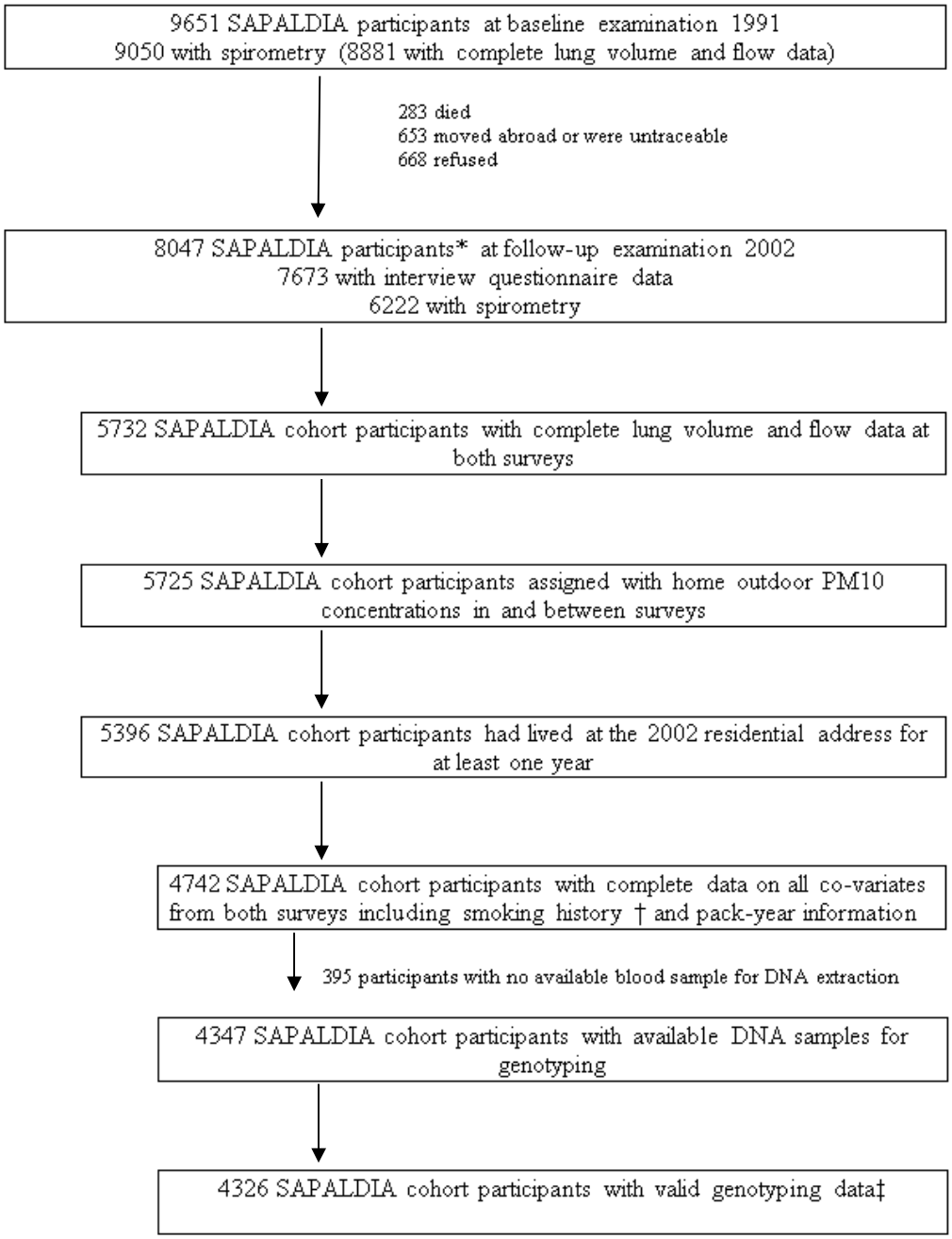
† Appendix 1

Supplement Material to „Improved PM_{10} Exposure Attenuates Age-Related Lung Function Decline: Genetic Variants in p53, p21 and CCND1 Modify this Effect"

Outline of Sections

<u>Supplement Material, Figure 1:</u> SAPALDIA cohort and study sample



Footnotes to Supplement Material, Figure 1

* provided self-completed screening questionnaire or interview administered questionnaire, spirometry and/or blood sample.

† 73 participants who reported being never or former smoker at either interview but had expired carbon monoxide >10ppm at the same interview were excluded.

‡ No successful genotypes were obtained in samples of 21 participants

Supplement Material, Table 1: Linkage disequilibrium and estimated haplotype frequencies of *CCND1* -7006G>C, rs667515, and *CCND1* P2424, rs9344.

A

Genotype distribution of *CCND1* rs9344 and rs667515, P2424. (P<0.001)

		rs9344:		
		GG	**GC**	**CC**
rs667515:	**GG**	113	590	925
	GA	498	1513	47
	AA	600	37	3

B

Estimated *CCND1* haplotype frequency (%)

Haplotype*		**E(freq)%**	**S.E**
Haplotype 1	(G-G)	15.4	0.0492
Haplotype 2	(G-A)	46.3	0.0488
Haplotype 3	(C-G)	37.1	0.0493
Haplotype 4	(C-A)	1.2	0.0483

*Haplotypes were constructed using PHASE 2.0; order of CCND1 SNPs: *CCND1* -7006G>C (rs667515), *CCND1* P242P G>A (rs9344).

Supplement Material, Table 2: Characteristics of the study population and comparison to non-participants, the SAPALDIA cohort.

	Participants Included in Analysis	Participants Excluded from Analysis[a]	Non-participants[b]	P-value included vs. excluded	P-value included vs. non-participants
Number	N=4326	N=1415	N=3309		
Female [%]	53.0	52.2	49.2	0.610	0.001
Age in 1991 (mean, SD) [yrs]	41.3 (11.2)	39.6 (11.7)	41.2 (12.1)	<0.001	0.686
Height (mean, SD) [cm]	169.3 (8.8)	169.5 (8.8)	168.9 (9.2)	0.472	0.065
BMI in 1991 (mean, SD) [kg/m2]	23.7 (3.6)	23.8 (3.7)	24.4 (4.2)	0.412	<0.001
BMI change (mean, SD) [kg/m2]	2.1 (2.2)	2.0 (2.4)	na	0.202	na
Never smokers in 1991 [%]	48.4	42.1	38.5	<0.001	<0.001
Never smokers in 2002 [%]	49.3	35.0	na	<0.001	na
Smoking quitters during follow-up [%]	8.1	12.8	na	<0.001	na
Current smokers in 1991 [%]	29.3	32.1	39.6	0.047	<0.001
Current smokers in 2002 [%]	21.9	33.9	na	<0.001	na
Number of pack years for current smokers in 2002 (median, IQR)	26.7 (14.0 to 42.6)	20.6 (11.4 to 39.0)	na	0.002	na
Cigarettes per day for current smokers in 1991 (median, IQR)	20 (10 to 25)	20 (10 to 20)	20 (12 to25)	<0.001	0.469
Cigarettes per day in current smokers in 2002 (median, IQR)	15 (7 to 20)	10 (2 to 20)	na	<0.001	na
ETS exposure in never smokers in 1991 [%]	13.1	13.7	12.5	0.613	0.390
ETS exposure in never smokers in 2002 [%]	7.7	4.5	na	<0.001	na
Father or mother smoked during childhood[%]	56.1	56.3	59.3	0.883	0.005
Workplace exposure to dust and fumes in 1991 [%]	30.3	29.8	33.6	0.720	0.002
Workplace exposure to dust and fumes in 2002	13.2	15.5	na	0.037	

[%]					
Atopy in 1991 [%]	22.3	25.7	23.4	0.010	0.285
Swiss nationality [%]	87.7	85.7	74.7	0.046	<0.001
Educational level in 2002 (professional education or higher) [%]	27.9	30.5	na	0.147	na
Increase in educational level between surveys [%]	17.9	19.4	na	0.194	na

Footnotes to Spplement Material, Table 2

[a] Excluded participants had spirometry performed at both visits, but were not included in the final analysis because of missing covariate or genotype data.

[b] Non-participants had spirometry performed at baseline in 1991, but either did not have spirometry performed at follow-up in 2002 or did not at all participate in the follow-up assessment in 2002.

Abbreviations: BMI - body mass index, ETS - environmental tobacco smoke, IQR - inter quartile range, SD - standard deviation.

Main effect estimates of genetic variants

The main effects are presented in Supplement Material, Table 3. No statistically significant associations of the polymorphisms with either change in FEF_{25-75} or with FEV1 or FVC were observed. The effect of the combination of both *CCND1* SNPs on change in lung function was assessed after inference of haplotype frequency and diplotype distribution. The two *CCND1* SNPs are in high linkage disequilibrium (Lewtonin's D'= 0.93 and r^2=0.49); a frequency table of the four possible haplotypes is presented in Supplement Material, Table 1. None of the CCND1 haplotypes was associated with lung function decline irrespective of the lung function parameter studied (data not shown).

Supplement Material, Table 3: Main effects of genotypes: Association[a] of genetic variation in *p53*, *p21* and *CCND1* with average annual lung function decline.

Genotype		N	Average difference in annual decline in lung function (mL/y) relative to the reference genotype[b]	95% confidence interval		P[c]
p53 **Pro72Arg; rs1042522**						
GG	**FVC**	2407	ref.			
CG		1637	-0.43	-2.62	1.77	0.704
CC		282	+1.13	-3.20	5.46	0.610
GG	**FEV1**		ref.			
CG			-0.83	-2.48	0.82	0.324
CC			+0.20	-3.06	3.46	0.904
GG	**FEF25_75**		ref.			
CG			-1.45	-5.23	2.33	0.451
CC			-3.09	-10.54	4.35	0.415
p21 **Ser31Arg; rs1801270**						
CC	**FVC**	3701	ref.			
CA or AA[d]		625	+1.20	-1.78	4.18	0.429
CC	**FEV1**		ref.			
CA or AA[d]			+0.59	-1.64	2.82	0.605
CC	**FEF25_75**		ref.			
CA or AA[d]			-0.21	-5.31	4.90	0.937

***CCND1* P242P; rs9344**						
GG	**FVC**	1211	ref.			
AG		2140	+2.32	-0.15	4.79	0.066
AA		975	+1.23	-1.73	4.19	0.416
GG	**FEV1**		ref.			
AG			-0.03	-1.88	1.82	0.974
AA			-0.16	-2.38	2.06	0.888
GG	**FEF25_75**		ref.			
AG			-3.94	-8.17	0.30	0.068
AA			-4.58	-9.65	0.50	0.077
***CCND1* -7006G>C; rs667515**						
GG	**FVC**	1628	ref.			
CG		2058	+0.66	-1.62	2.94	0.569
CC		640	-2.59	-5.80	0.63	0.114
GG	**FEV1**		ref.			
CG			+0.53	-1.18	2.24	0.545
CC			-0.48	-2.89	1.94	0.699
GG	**FEF25_75**		ref.			
CG			+2.31	-1.60	6.23	0.247
CC			+3.72	-1.79	9.23	0.186

Footnotes to Supplement Material, Table 3

[a] Covariates included age, age^2, sex, height, parental smoking, sine and cosine function of day of examination to control for seasonal effects, level of education at SAPALDIA 1, change in level of education, Swiss nationality, self reported occupational exposure to dust and occupational exposure to fumes at SAPALDIA 1 and SAPALDIA 2 (yes/no), smoking status at SAPALDIA 2 (never, former or current), pack years up to SAPALDIA 1, pack years between SAPALDIA 1 and 2, cigarettes per day at SAPALDIA 1 and SAPALDIA 2, atopy, BMI at SAPALDIA 1, change in BMI, interaction between the two BMI variables, baseline PM_{10} exposure and annual change in PM_{10}.

[b] Positive estimates: annual lung function decrease smaller than in the reference category. Negative estimates: annual lung function decrease larger than in the reference category.

[c] Bonferroni significance level for twelve comparisons (three respiratory function tests (FVC, FEV1, FEF25-75) times four association tests) P=0.00417.

[d] Genotype distribution: *p21* CA: N=594; *p21* AA: N=31.

Abbreviations: BMI - body mass index, CCND1 - Cyclin D1, FEF25-75 - forced expiratory flow between 25 and 75% of FVC, p21 - Cyclin dependant kinase inhibitor A1, also known as Waf1 or Cip1, P242P - proline to proline substitution at amino acid 242, P242P- proline to proline substitution at amino acid 242, p53 - tumor protein p53, PM_{10} - particulate matter of less than 10 µm aerodynamic diameter, R72P - arginine to proline substitution at amino acid 72, S31R - serine to arginine substitution at amino acid 31.

[e] Diplotype distribution was labeled as followed: "-/-" stands for none of the specific haplotype present; "*(rs667515, rs9344)* /-" as in e.g. "GG/-" stands for one of the specific haplotype present; "*(rs667515, rs9344)* / *(rs667515, rs9344)* " as in e.g. "GG/GG" stands for two of the specific haplotypes present.

Abbreviations: BMI - body mass index, CCND1 - Cyclin D1, FEF25-75 - forced expiratory flow between 25 and 75% of FVC, p21 - Cyclin dependant kinase inhibitor A1, also known as Waf1 or Cip1, P242P - proline to proline substitution at amino acid 242, P242P- proline to proline substitution at amino acid 242, p53 - tumor protein p53, PM_{10} - particulate matter of less than 10 µm aerodynamic diameter, R72P - arginine to proline substitution at amino acid 72, S31R - serine to arginine substitution at amino acid 31.

<u>Supplement Material, Table 4:</u> Effect modification by genotypes: Association[a] of change in average home outdoor PM_{10} (per decrease of 10 µg/m3 between 1991 and 2002) with average annual change in lung function, by genotype status, among never smoking participants.

Genotypes	N	Outcome	Average annual lung function decline (mL/y) associated with 10 µg/m^3 PM10 decrease during follow-up[b]	95% Confidence Interval		P	Pinteraction[c]
***p53* R72P; rs1042522**							
GG	1145	**FVC**	1.66	-5.42	8.73	0.646	$0.613_{(recessive)}$
CG	758		3.4	-3.84	10.64	0.358	
CC	135		-0.57	-12.53	11.38	0.925	
GG		**FEV1**	4.65	-0.85	10.15	0.097	$0.084_{(recessive)}$
CG			7.03	1.4	12.67	**0.014**	
CC			-2.39	-11.68	6.9	0.614	
GG		**FEF25_75**	17.41	4.92	29.91	**0.006**	$0.015_{(recessive)}$
CG			18.9	6.1	31.7	**0.004**	
CC			-7.91	-29.05	13.23	0.463	
***p21* S31R; rs1801270**							
CC	1738	**FVC**	2.74	-3.27	8.75	0.371	$0.423_{(dominant)}$
CA/AA[d]	300		-1.81	-13.58	9.97	0.764	
CC		**FEV1**	5.22	0.55	9.89	**0.029**	$0.315_{(dominant)}$
CA/AA[d]			0.79	-8.36	9.95	0.865	
CC		**FEF25_75**	14.29	3.68	24.9	**0.008**	$0.538_{(dominant)}$
CA/AA[d]			20.46	-0.37	41.3	**0.054**	
***CCND1* P242P; rs9344**							
GG	577	**FVC**	-2.54	-10.96	5.88	0.555	$0.116_{(dominant)}$
AG	984		4.51	-2.6	11.63	0.214	
AA	477		2.74	-5.29	10.77	0.503	
GG		**FEV1**	4.07	-2.49	10.63	0.224	$0.248_{(recessive)}$
AG			6.99	1.44	12.53	**0.014**	
AA			2.31	-3.94	8.57	0.468	
GG		**FEF25_75**	22.61	7.74	37.48	**0.003**	$0.032_{(recessive)}$

AG			17.83	5.26	30.41	**0.005**	
AA			4.33	-9.86	18.52	0.55	
***CCND1* -7006G>C; rs667515**							
GG	782	**FVC**	0.86	-6.01	7.74	0.806	$0.446_{(dominant)}$
CG	968		4.69	-2.74	12.12	0.216	
CC	288		1.01	-10	12.01	0.858	
GG		**FEV1**	3.28	-2.07	8.63	0.230	$0.103_{(co\text{-}dominant)}$
CG			5.39	-0.4	11.17	0.068	
CC			10.86	2.29	19.43	**0.013**	
GG		**FEF25_75**	12.67	0.52	24.83	**0.041**	$0.041_{(recessive)}$
CG			13.42	0.28	26.55	**0.045**	
CC			32.02	12.55	51.49	**0.001**	
***CCND1* Haplotype 1 (rs667515, rs9344)** [e]							
-/-	1461	**FVC**	3.89	-2.59	10.36	0.239	$0.165_{(co\text{-}dominant)}$
GG/-	519		-0.37	-8.44	7.7	0.928	
GG/GG	58		-8.69	-31.64	14.27	0.458	
-/-		**FEV1**	5.61	0.57	10.66	**0.029**	$0.440_{(dominant)}$
GG/-			3.13	-3.16	9.41	0.329	
GG/GG			4.53	-13.34	22.4	0.619	
-/-		**FEF25_75**	13.23	1.78	24.69	**0.024**	$0.277_{(recessive)}$
GG/-			16.52	2.24	30.8	**0.023**	
GG/GG			36.52	-4.16	77.19	0.078	
***CCND1* Haplotype 2 (rs667515, rs9344)** [e]							
-/-	591	**FVC**	-0.94	-9.18	7.3	0.822	$0.274_{(dominant)}$
GA/-	997		4.18	-3.04	11.4	0.256	
GA/GA	450		2.17	-5.88	10.22	0.597	
-/-		**FEV1**	5.84	-0.58	12.26	0.075	$0.182_{(recessive)}$
GA/-			6.29	0.66	11.91	**0.028**	
GA/GA			1.91	-4.36	8.19	0.550	
-/-		**FEF25_75**	23.18	8.62	37.73	**0.002**	$0.030_{(co\text{-}dominant)}$
GA/-			17.31	4.54	30.07	**0.008**	
GA/GA			4.47	-9.77	18.71	0.538	
***CCND1* Haplotype 3 (rs667515, rs9344)** [e]							

-/-	809	**FVC**	1.17	-5.7	8.04	0.738	$0.333_{(recessive)}$
CG/-	955		4.9	-2.42	12.22	0.189	
CG/CG	274		-2.38	-13.98	9.22	0.687	
-/-		**FEV1**	3.5	-1.85	8.86	0.200	$0.304_{(co\text{-}dominant)}$
CG/-			6.17	0.46	11.87	**0.034**	
CG/CG			7.4	-1.64	16.44	0.108	
-/-		**FEF25_75**	12.72	0.57	24.87	**0.040**	$0.060_{(recessive)}$
CG/-			14.51	1.57	27.45	**0.028**	
CG/CG			31.95	11.42	52.48	**0.002**	
***CCND1* Haplotype 4 (rs667515, rs9344)** [e]							
-/-	1999	**FVC**	1.5	-4.45	7.45	0.621	$0.015_{(co\text{-}dominant)}$
CA/-	37		35.22	7.91	62.53	**0.012**	
CA/CA	2		94.98	-689.8	879.77	0.812	
-/-		**FEV1**	4.07	-0.56	8.7	0.085	$0.003_{(co\text{-}dominant)}$
CA/-			35.98	14.75	57.21	**0.001**	
CA/CA			3.63	-606.34	613.61	0.991	
-/-		**FEF25_75**	14.81	4.27	25.34	**0.006**	$0.984_{(recessive)}$
CA/-			15.00	-33.45	63.45	0.544	
CA/CA			27.53	-1364.56	1419.62	0.969	

Footnotes to Supplement Material, Table 4

[a] Covariates included age, age^2, sex, height, parental smoking, sine and cosine function of day of examination to control for seasonal effects, level of education at SAPALDIA 1, change in level of education, Swiss nationality, self reported occupational exposure to dust and occupational exposure to fumes at SAPALDIA 1 and SAPALDIA 2 (yes/no), smoking status at SAPALDIA 2 (never, former

or current), pack years up to SAPALDIA 1, pack years between SAPALDIA 1 and 2, cigarettes per day at SAPALDIA 1 and SAPALDIA 2, atopy, BMI at SAPALDIA 1, change in BMI, interaction between the two BMI variables and baseline PM_{10} exposure.

b Positive estimates: attenuation of lung function decline due to PM_{10} decrease. Negative estimates: acceleration of lung function decline due to PM_{10} decrease.

c Pint: P-value for interaction between change in home outdoor exposure of PM_{10} and genotype parameterized in three different genetic models. The p-value for interaction presented in the table represents the most significant (lowest) p-value obtained for the three different genetic models.

d Genotype distribution in never smokers: *p21* CA: N=288; *p21* AA: N=12.

e Diplotype distribution was labeled as followed: "-/-" stands for none of the specific haplotype present; "*(rs667515, rs9344)* /-" as in e.g. "GG/-" stands for one of the specific haplotype present; "*(rs667515, rs9344)* / *(rs667515, rs9344)* " as in e.g. "GG/GG" stands for two of the specific haplotypes present

Abbreviations: BMI - body mass index, CCND1 - Cyclin D1, FEF25-75 - forced expiratory flow between 25 and 75% of FVC, p21 - Cyclin dependant kinase inhibitor A1, also known as Waf1 or Cip1, P242P - proline to proline substitution at amino acid 242, P242P- proline to proline substitution at amino acid 242, p53 - tumor protein p53, PM_{10} - particulate matter of less than 10 µm aerodynamic diameter, R72P - arginine to proline substitution at amino acid 72, S31R - serine to arginine substitution at amino acid 31.

Supplement Material, Table 5: Effect modification by genotypes: Association[a] of change in average home outdoor PM_{10} (per decrease of 10 µg/m3 between 1991 and 2002) with average annual change in FEV1 and FVC, by genotype status.

Genotypes	N	Outcome	Average annual lung function decline (mL/y) associated with 10 µg/m^3 PM10 decrease during follow-up[b]	95% Confidence interval		P	P interaction[c]
***p53* R72P; rs1042522**							
GG	2407		0.95	-3.95	5.86	0.703	$0.424_{(dominant)}$
CG	1637	**FVC**	-1.39	-6.62	3.84	0.603	
CC	282		0.16	-9.75	10.08	0.974	
GG			4.89	1.21	8.57	0.009	$0.105_{(co\text{-}dominant)}$
CG		**FEV1**	2.88	-1.05	6.81	0.151	
CC			-1.13	-8.58	6.32	0.767	
***p21* S31R; rs1801270**							
CC	3701	**FVC**	0.27	-4.04	4.58	0.902	$0.562_{(dominant)}$
CA or AA[d]	625		-2.12	-10.66	6.41	0.626	
CC		**FEV1**	3.71	0.48	6.94	0.024	$0.764_{(dominant)}$
CA or AA[d]			4.64	-1.77	11.04	0.156	
***CCND1* P242P; rs9344**							
GG	1211		-1.32	-7.61	4.96	0.679	$0.632_{(dominant)}$
AG	2140	**FVC**	0.34	-4.53	5.21	0.891	
AA	975		-0.36	-6.52	5.8	0.908	
GG			4.75	0.04	9.46	0.048	$0.164_{(recessive)}$
AG		**FEV1**	4.18	0.53	7.83	0.025	
AA			1.2	-3.42	5.82	0.61	
***CCND1* -7006G>C; rs667515**							
GG	1628		-1.71	-7.01	3.58	0.526	$0.232_{(co\text{-}dominant)}$
CG	2058	**FVC**	0.33	-4.68	5.34	0.897	
CC	640		2.84	-4.88	10.56	0.47	
GG			1.45	-2.53	5.42	0.476	$0.007_{(co\text{-}dominant)}$
CG		**FEV1**	3.57	-0.19	7.33	0.062	

CC			10.23	4.44	16.02	0.001	
***CCND1* Haplotype 1 (rs667515, rs9344)** [e]							
-/-	3125		0.7	-3.78	5.18	0.76	$0.131_{(co\text{-}dominant)}$
GG/-	1088	**FVC**	-2.49	-8.81	3.84	0.441	
GG/GG	113		-13.94	-34.08	6.19	0.175	
-/-			4.28	0.92	7.64	0.013	$0.169_{(dominant)}$
GG/-		**FEV1**	1.17	-3.58	5.91	0.63	
GG/GG			0.97	-14.14	16.08	0.9	
***CCND1* Haplotype 2 (rs667515, rs9344)** [e]							
-/-	1251		-0.29	-6.47	5.89	0.926	$0.834_{(recessive)}$
GA/-	2150	**FVC**	0	-4.9	4.9	1	
GA/GA	925		-0.68	-6.85	5.5	0.83	
-/-			5.57	0.94	10.21	0.019	$0.092_{(co\text{-}dominant)}$
GA/-		**FEV1**	3.9	0.22	7.58	0.038	
GA/GA			0.88	-3.75	5.52	0.709	
***CCND1* Haplotype 3 (rs667515, rs9344)** [e]							
-/-	1678		-1.49	-6.79	3.8	0.58	$0.393_{(dominant)}$
CG/-	2048	**FVC**	0.63	-4.34	5.6	0.804	
CG/CG	600		1.1	-6.88	9.09	0.787	
-/-			1.61	-2.36	5.58	0.427	$0.022_{(co\text{-}dominant)}$
CG/-		**FEV1**	3.86	0.13	7.59	0.043	
CG/CG			9.05	3.06	15.05	0.003	
***CCND1* Haplotype 4 (rs667515, rs9344)** [e]							
-/-	4239		-0.54	-4.79	3.71	0.803	$0.019_{(dominant)}$
CA/-	84	**FVC**	27.4	5.1	49.7	0.016	
CA/CA	3		-85.56	-351.48	180.36	0.528	
-/-			3.25	0.06	6.44	0.046	$0.014_{(dominant)}$
CA/-		**FEV1**	25.21	8.48	41.94	0.003	
CA/CA			-66.31	-265.82	133.21	0.515	

Footnotes to Supplement Material, Table 5

[a] Covariates included age, age^2, sex, height, parental smoking, sine and cosine function of day of examination to control for seasonal effects, level of education at SAPALDIA 1, change in level of education, Swiss nationality, self reported occupational exposure to dust and occupational exposure to fumes at SAPALDIA 1 and SAPALDIA 2 (yes/no), smoking status at SAPALDIA 2 (never, former or current), pack years up to SAPALDIA 1, pack years between SAPALDIA 1 and 2, cigarettes per day at SAPALDIA 1 and SAPALDIA 2, atopy, BMI at SAPALDIA 1, change in BMI, interaction between the two BMI variables and baseline PM_{10} exposure.

[b] Positive estimates: attenuation of lung function decline due to PM_{10} decrease. Negative estimates: acceleration of lung function decline due to PM_{10} decrease.

[c] Pinteraction: P-value for interaction between change in home outdoor exposure of PM_{10} and genotype parameterized in three different genetic models. The p-value for interaction presented in the table represents the most significant (lowest) p-value obtained from the three different genetic models. Bonferroni significance level for twelve comparisons (three respiratory function tests (FVC, FEV1, FEF25-75) times four association tests) P=0.00417.

[d] Genotype distribution: *p21* CA: N=594; p21 AA: N=31.